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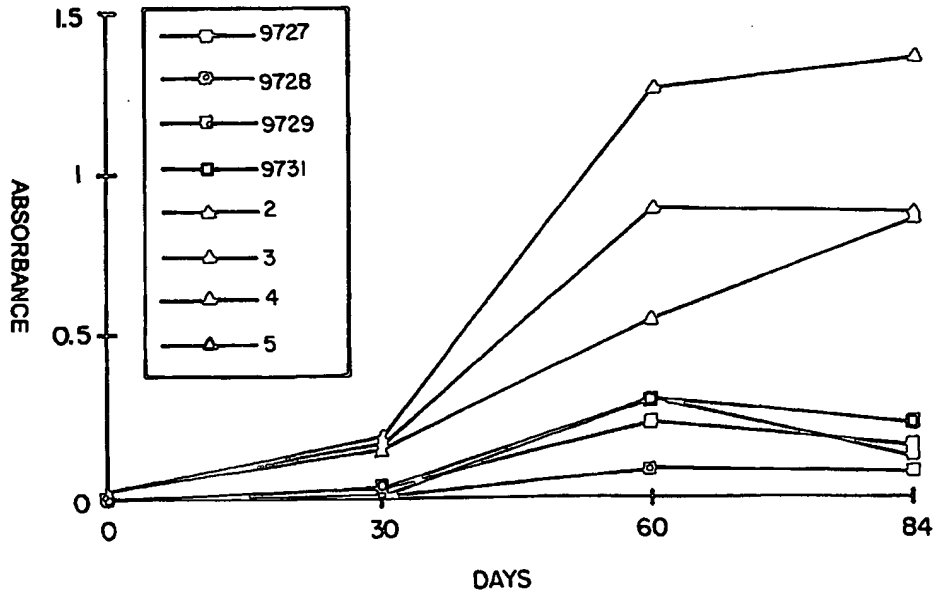

(57) Abstract

A vaccine comprising an antigen derived from a zona pellucida glycoprotein is effective to impair fertility in animals, preferably carnivores. The vaccine can be used as an immunosterilant or an immunocontraceptive.

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FERTILITY IMPAIRING VACCINE AND METHOD OF USE

This application claims the benefit of U.S. Provisional Application No. 60/070,375, filed January 2, 1998, U.S. Provisional Application No. 60/071,406, filed January 15, 1998, and U.S. Provisional Application No. 60/076,368, filed February 27, 1998.

Background of the Invention

Traditional methods of population control in dogs have been unsuccessful. Surgical spaying is a laborious procedure, requiring the initial induction of the animal, gas anesthesia during surgery, a surgical pack with suture materials and post-operative medications. Common surgical complications include problems associated with the procedure itself, allergic reactions to anesthetics or post-operative medications, and adverse local or systemic effects during the recovery period. Examples include ovarian remnant syndrome, where dogs continue to cycle despite being spayed, uterine infections, abdominal hemorrhage, and premature opening of the suture line. A substantial recovery period is typically needed even after an uncomplicated procedure. Surgical spaying is also expensive, and pet owners are often unwilling to assume the costs.

Hormonal therapies have also been used to curb pet overpopulation. However these methods usually require daily administration of the drug, and they only result in temporary infertility. Furthermore, most protracted hormonal therapies have undesirable side effects such as uterine infections, mammary cancer, and diabetes.

Previous studies (e.g., C. Mahi-Brown et al., J. Exp. Zool., 222, 89-95 (1982)) have shown that fertility impairment in the female dog can be achieved by vaccinating with a preparation containing a glycoprotein associated

with the mammalian egg, namely the pig zona pellucida (pZP). The vaccine contained a crude extract of porcine zona pellucida, obtained via collegenase digestion of ovarian material to remove follicular cells and an adjuvant, namely Freund's Complete adjuvant, alum adjuvant, or CP-20,961 (C. Mahi-Brown et al., Biol. Reprod., 32, 761-772 (1985)). Collegenase treatment of zona pellucida proteins is known to alter the proteins in a way that can be demonstrated immunocytochemically. Abnormal estrus cycles, characterized by constant or prolonged estrus, and other deleterious side effects, such as ovarian cyst formation, were found to be associated with the vaccinations (C. Mahi-Brown, Am. J. Reprod. Immunol. Microbiol., 18, 94-103 (1988)), and were never satisfactorily explained.

A vaccine comprising porcine zona pellucida and an adjuvant comprising synthetic trehalose dicorynomycolate has been successfully used to cause immunocontraception in horses (P. Willis et al., J. Equine Vet. Sci., 364-370 (1994)) and elephants (R. F-H., Wildlife Soc. Bull., 25(1):18-21 (1997)). Dunbar et al. (e.g., EP 599822, U.S. Pat. No. 5,637,300) have experimented with reproductive control in non-rodent mammals using a recombinant zona pellucida protein. Due to limitations imposed by recombinant DNA technology and available expression systems, however, the recombinant protein lacks the glycosylation pattern of the native glycoprotein.

In humane shelters population control of unwanted pets is currently achieved through euthanasia of the animals. In general, after capture, dogs are held for a period of one week. If they are not adopted, they are humanely destroyed.

There is, therefore, a demonstrated need for a safe, simple method for sterilizing animals, particularly cats and dogs, that is both permanent and relatively inexpensive.

Summary of the Invention

The present invention provides a vaccine and a method for impairing fertility in an animal. The method for impairing fertility in the animal comprises administering to the animal a vaccine comprising substantially pure, nonrecombinant zona pellucida glycoprotein, or an antigenic fragment thereof. The vaccine is administered in a manner and an amount effective to cause fertility impairment in the animal. When administered as an immunocontraceptive, the fertility impairment vaccine causes temporary, reversible infertility in the animal. When administered as an immunosterilant, the fertility impairing vaccine causes permanent, irreversible infertility in the animal. Preferably, the animal to which the vaccine is administered is a carnivore. Preferably, the carnivore is a dog or a cat; more preferably, the carnivore is a dog. The vaccine preferably does not cause abnormal estrus cycles in a vaccinated dog.

The fertility impairing vaccine of the invention preferably comprises porcine zona pellucida glycoprotein, and optionally includes an immunological adjuvant comprising an immunostimulant, preferably synthetic trehalose dicorynomycolate (STDCM). Also optionally, the vaccine contains an oil, preferably squalene oil.

In a preferred embodiment, the fertility impairing vaccine is an immunosterilant vaccine. The immunosterilization method of the invention is far preferable to surgical sterilization and hormone regimens as a population control tool for domestic dogs and cats, and can further be used to control ferrel dog and cat populations, for example by development of a species-specific oral delivery vehicle.

Brief Description of the Drawings

Figure 1 shows one version of the oocyte purification apparatus of the invention.

Figure 2 is a graph depicting serum anti-porcine zona pellucida antibody titers in experimental dogs (subjects 9727, 9728, 9729 and 9731) and clinical dogs (subjects 2-5) during the course of vaccination with a porcine zona pellucida (pZP) vaccine.

Detailed Description of the Preferred Embodiments

The fertility impairing vaccine of the invention comprises an antigen comprising zona pellucida glycoprotein, preferably substantially pure zona pellucida glycoprotein, or an antigenic fragment thereof. Preferably, zona pellucida glycoprotein is a total porcine zona pellucida glycoprotein. A total zona pellucida glycoprotein preparation obtained from pig ovaries includes all three major heavily glycosylated porcine zona pellucida glycoproteins: pZP1, pZP3α and pZP3β. pZP3α and pZP3β each have reported molecular weights of about 55 kD, and pZP1 has a reported molecular weight of about 82 kD. The amino acid sequences of these three glycoproteins are known (J.D. Harris et al., DNA Seq., 4, 361-393 (1994)). Other reported pZP glycoproteins are believed to be degradation products of pZP1.

Purity of the zona pellucida glycoprotein can be evaluated analytically using a combination or series of two-dimensional sodium dodecyl sulfate polyacrylamide gels (SDS-polyacrylamide gel electrophoresis, or SDS-PAGE) with silver staining, Coomassie Blue staining, and Western blot analysis, as described in the following Examples. Glycoproteins typically migrate electrophoretically in gels as broad smears rather than narrow bands, as a result of the variable levels of negative charge present in the constituent oligosaccharide chains. A "substantially pure" total zona pellucida glycoprotein preparation isolated from pig ovaries migrates as two distinct smears in the gel

electrophoretic experiments (one smaller smear representing pZP1, and one larger smear representing pZP3α and pZP3β), and shows immunological reactivity in Western blot analysis using a polyclonal antibody raised in rabbits to highly purified total porcine zona pellucida glycoprotein. In a substantially pure zona pellucida glycoprotein preparation used for fertility impairment, there are no detectable contaminating proteins. The absence of detectable contaminating proteins is determined by demonstrating that there are no proteins in the preparation that have electromigration patterns different from those exhibited by the zona pellucida glycoproteins as determined by two-dimensional SDS-PAGE (silver-stained) or Western blot analyses of two-dimensional SDS-PAGE gels. An antigenic fragment of a zona pellucida glycoprotein is a peptide fragment, preferably a glycosylated peptide fragment, that elicits an immune response characterized by detectable anti-pZP antibody levels in the subject using ELISA as described in Example II. The peptide fragment preferably contains more than seven amino acids, more preferably at least about 10 amino acids, most preferably at least about 20 amino acids.

The zona pellucida glycoprotein used in the present vaccine is preferably a naturally occurring glycoprotein or a chemically or enzymatically synthesized glycoprotein. The glycoprotein is preferably not a recombinant glycoprotein, but use of a recombinant glycoprotein in the present vaccine is not necessarily excluded in alternative embodiments of the invention.

The vaccine of the invention preferably additionally includes an immunological adjuvant to enhance the immunological response of the subject to the glycoprotein antigen. Examples of adjuvants include Freund's Complete Adjuvant, Freund's Incomplete Adjuvant, and an adjuvant comprising an immunostimulant such as synthetic trehalose dicorynomycolate (STDCM) and an oil such as squalene oil (see P. Willis et al., J. Equine Vet. Sci., 14, 364-370 (1994)). An adjuvant comprising synthetic trehalose dicorynemycolate, squalene oil, and a surfactant such as lecithin is preferred. Lecithin typically includes phosphatidyl choline.

In a preferred embodiment the vaccine comprises oil, preferably a biodegradable oil such as squalene oil, in an amount of about 2.5% to about 15%, preferably about 8% to about 12%. In preparing the vaccine it is advantageous to combine a concentrated oily adjuvant composition with an aqueous solution of the antigen, pZP glycoprotein. Typically, the vaccine is prepared using an adjuvant concentrate which contains lecithin (about 5% to about 15 % wt/vol, preferably about 12% wt /vol) and STDCM (preferably about 25 mg/mL to about 50 mg/mL) in squalene oil. The term % wt/vol means grams per 100 mL of liquid. The aqueous solution containing the isolated pZP glycoprotein is typically a phosphate-buffered saline (PBS) solution, and additionally preferably contains Tween 80 (about 0.2% vol/vol to about 0.8% vol/vol, preferably about 0.4% vol/vol). See J.A. Rudbach et al., "Ribi Adjuvants: Chemistry, Biology and Utility in Vaccines for Human and Veterinary Medicine," in The Theory and Practical Application of Adjuvants, D.E.S. Stewart-Tull, Ed., John Wiley & Sons, New York, NY (1995)). Homogenization of the oily adjuvant concentrate with the aqueous pZP solution can be accomplished using any convenient means known in the art, such that the oil disperses within the aqueous solution to form an oil in water emulsion. Oil droplet sizes of about 200 nm or less are particularly preferred as they produce a more uniform and stable suspension. A particularly preferred vaccine comprises predetermined amounts of pZP and STDCM in an emulsion containing about 10% squalene oil and about 90% aqueous phase.

The invention further includes a method for administering a fertility impairing vaccine as described herein in a manner effective to cause impaired fertility in an animal, preferably a carnivore (i.e., a member of the order *Carnivora*). Preferably the carnivore is not a primate, and is a dog or a cat, more preferably a dog. Impairment of fertility in an animal in accordance with the invention can take the form of either immunocontraception and immunosteriliztion. Immunosterilization means permanent, irreversible infertility, in contrast to immunocontraception wherein infertility is temporary or transient, and reversible. Immunocontraception and immunosterilization are

both dependent on the antibody titer level in the serum of the subject, but immunosterilization is typically the result of ovarian pathology caused by vaccine administration and high titers of anti-pZP antibodies, as evidenced by, for example, total destruction of the zona pellucida glycoproteins and/or influx of leukocytes into the follicles. Reducing the number of boosters leads to lower antibody titers which results in immunocontraception (i.e., infertility that is temporary and reversible) instead of immunosterilization.

The vaccine is administered in a manner and an amount effective to cause the desired infertility in the mammalian subject. For example, to immunosterilize a dog or a cat, the vaccine is preferably administered in the form of a plurality of doses (typically about 1.0 mL for a dog, 0.5 mL for a cat), each dose containing zona pellucida glycoprotein, or an antigenic fragment thereof, in an amount of about 100 μg to about 2 mg, more preferably about 200 μg to about 400 μg. An immunostimulant such as STDCM is typically present in a per dose amount of about 50 μg to about 5 mg, preferably in an amount of about 1 mg to about 3.5 mg, more preferably in an amount of about 2 mg to about 3 mg. The animal is given an initial dose, usually via intramuscular injection although subcutaneous injection can also be used. The initial injection is followed by two or more booster injections at two to four week intervals, although the boosters can be administered from about 9 days to about twelve months following the previous vaccination. The body's immunological response to the vaccine at this dosing regimen appears to render the ovaries permanently inactive as a result of, for example, follicle disruption or destruction, as evidenced by immunocytochemical analysis and histological evaluation of the ovarian tissue of vaccinated subjects. Sterility is permanent and irreversible. Immunosterilization of carnivores in accordance with the present method typically does not cause abnormal estrus cycles or other significant undesirable side effects in the vaccinated subjects.

When the vaccine is administered to a dog or a cat as described above, but with only one booster instead of two or more boosters, the vaccine typically results in immunocontraception (i.e., temporary or transient, reversible infertility) rather than immunosterilization.

EXAMPLES

Advantages of the invention are illustrated by the following examples. However, the particular materials and amounts thereof recited in these examples, as well as other conditions and details, are to be interpreted to apply broadly in the art and should not be construed to unduly restrict or limit the invention in any way.

Example I. Isolation of Porcine Zona Pellucidae and Extraction of pZP Glycoproteins

Buffers. Saline buffer (40 L) was made by addition 4 L of the following solution: 0.9% NaCl, 0.01 M dibasic sodium phosphate, 0.01 M monobasic sodium phosphate, and 0.002 M sodium citrate dihydrate, pH 7.2, in triple distilled water, to 36 L of triple distilled water. Tris buffer (3L) was made by adding 484 g Tris base, 119 g ethylenediaminetetraacetic acid (EDTA), 47 g sodium citrate dihydrate and 16 g sodium azide to 3L of triple distilled water, then adjusting the pH to 7.9. Tris detergent buffer (1L) was made by combining 2 mL of NP-40 (Cat. No. N-6507, Sigma Chemical Co., St. Louis, MO) with 998 mL Tris buffer.

Other materials. The oocyte purification apparatus (Fig. 1) consisted of three chambers. Each chamber consisted of a stainless steel wire mesh container (Home Depot) suspended inside a buffer container set on an orbital shaker (shown in Fig. 1) or a rotary washing system with an internal agitator. The pore size of the wire mesh used to form the wire mesh containers in the first, second, and third chambers was 1000 μm, 500 μm, and 150 μm,

respectively. Tubing connecting the chambers allowed fluid transfer from the buffer space external to the wire mesh of one chamber to a collection or holding carboy, or, alternatively, to the inside of the next succeeding downstream wire mesh container in a continuous flow process, as shown in Fig. 1. Peristaltic pumps are used to effect fluid movement within the tubing between chambers (as shown in Fig. 1) or between the chambers and any collection carboys used (not shown in Fig. 1).

Pig ovaries were obtained from pig slaughterhouses.

Zona pellucida isolation. Porcine ovaries (5-6 lbs.) were twice ground through a commercial meat grinder (Hobart), and the homogenate was collected. The homogenate and grinder were rinsed with 4L of saline buffer, and the homogenate solution was placed in the wire mesh container of the first chamber of the purification apparatus. The three buffer containers of the purification apparatus were filled with saline buffer. The shakers were operated at an orbital shaker rotation speed of about 20 revolutions per minute during the oocyte purification process. Periods of rotary agitation were alternated with periods of fluid removal from the region surrounding the mesh container. Filtered oocytes, together with a small amount of tissue, passed through the 1000 μm mesh and were thus pumped from the buffer space of the first chamber into a collection carboy or into the wire mesh container in the second chamber. In purification procedures making use of a collection carboy, the filtered oocytes are subsequently pumped into the wire mesh container in the second chamber. With rotary agitation and new saline buffer addition, the oocytes were then passed through the 500 μm mesh of the wire mesh container of the second chamber while the fibrous tissue remained in the mesh container. The oocytes and saline buffer were then pumped from the buffer space of the second chamber into a collection carboy or directly into the 150 μm wire mesh container in the third chamber. Rotary agitation was continued in the third chamber and the solution surrounding the wire mesh (containing the oocytes) was removed.

The solution containing the oocytes was then passed over a 75 μm screen ($1^3/_4$ inches or 2 $_{1/2}$ inches in diameter). The oocytes were collected on the 75 μm screen and were then backwashed into a 100 mL beaker using Tris buffer. The 100 mL solution was divided into 2 x 50 mL vials and homogenized at 15,000 rpm for 3 to 5 minutes in a Powergen 700D (Fisher) homogenizer.

The zona fragments were then poured onto a $1^3/_4$ inches or 2 $_{1/2}$ inches diameter, 0.040mm (40μm) filter screen and washed with Tris detergent buffer. The zona fragments were removed from the screen by backwashing with Tris detergent buffer into a small polypropylene beaker, then incubated at 4°C with constant mechanical stirring to dissociate any undesired proteins, such as albumin. The zona material is preferably handled in polypropylene or siliconized glass beakers to prevent adherence to surfaces which results in loss of the material.

After incubation and stirring, the zona fragments were again poured a 1 $^3/_4$ inch diameter, 0.040mm (40μm) filter screen and washed with Tris buffer to remove any protein contaminants. The zona fragments retained on the screen were collected by spooning or backwashing (using Tris buffer) into a small polypropylene beaker to a maximal volume of 25 mL. The beaker was covered and placed in a 75-76°C water bath and incubated for 20 minutes to solubilize the zona protein such that the temperature of the zona protein-containing solution was 73 ± 1°C.

After solubilization, the mixture was centrifuged at 21,000 rpm for 25 minutes or until a pellet was observed at the base of the tube. The supernatant was collected, and protein concentration was estimated. The supernatant was aliquoted (3mg/vial), lyophilized, and stored under N_2 gas in a desiccator at 4°C. Typically about 1.5 mg to 1.9 mg of highly purified pZP protein per pound of ovaries can be produced, amounting to about 10 mg on a daily basis. Previous techniques produced only about 200 - 300 μg quantities over a two day period. It is anticipated that this harvesting technique of the present invention can be increased to produce even greater amounts.

Purity was demonstrated and confirmed using two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis combined with Western blot analysis, silver staining, and, at times, Coomassie blue staining, using standard protocols. The preparation was tested for viral and bacterial contaminants at the Diagnostic Laboratory at the College of Veterinary Medicine at the University of Georgia.

Example II. Preparation of pZP Vaccine

The vaccine was prepared by homogenizing a concentrated oily adjuvant concentrate with an aqueous antigen solution containing isolated pZP glycoprotein. The oily adjuvant concentrate contained a surfactant, lecithin, and an immunostimulant, synthetic trehalose dicorynomycolate (SDTCM), in squalene oil. A typical adjuvant concentrate contained about 12% wt/vol (grams/100 mL) lecithin and about 25-50 mg/mL STDCM in squalene oil. The aqueous antigen solution contained the pZP glycoprotein preparation in saline or phosphate buffered saline (PBS) and Tween 80. When prepared for use in combination with an adjuvant concentrate to yield the vaccine composition, the aqueous composition typically contained 0.4% (vol/vol) Tween 80 and an amount of pZP calculated to yield a dose of about 200 μg to about 400 μg per vaccination. Vaccine doses for dogs were about 1 mL in volume.

Homogenizing was accomplished by combining adjuvant concentrate (to a final concentration of no greater than 10% vol/vol) with aqueous pZP solution and emulsifying using a Powerjam 700D homogenizer at 15,000 rpm for 6 minutes. The resulting emulsion is then homogenized with phosphate buffered saline (PBS) (containing 0.4% vol/vol Tween 80) at 20,000 for 8-12 minutes. The homogenization process resulted in a vaccine composition that is an oil-in-water emulsion or possibly a water-in-oil-in-water emulsion. While the inventors do not intend that the invention be bound by any particular scientific theory, it is believed that the STDCM, an amphiphilic

glycolipid, partitions to the oil/water interfaces in the emulsion, and that the antigen is attracted to and associates with the STDCM at these interfaces.

Example III. Immunosterilization of Dogs using pZP Vaccine

Vaccinations. Four experimental dogs were vaccinated, and an FDA approved clinical trial has begun in which privately owned dogs in Clark and Walton Counties, Georgia, have also been vaccinated. To date, 43 dogs (four experimental dogs and 39 privately owned dogs) have been through the series of injections and have had serum antibody levels determined.

Female dogs were vaccinated with 200 μg of pZP per dose (1 mL) in a vaccine adjuvanted with synthetic trehalose dicorynomycolate (STDCM, commercially available from RIBI Immunochem Co., Hamilton, MT) in squalene oil. The amount of STDCM per dose was about 2.5 mg. An adjuvant concentrate as described in Example II was provided by RIBI Immunochem Co., Hamilton, MT, and the vaccines were prepared as described in Example II. The dogs were vaccinated consecutive boosters (containing the same amount of pZP, 200 μg) administered at 30-day intervals. Under veterinary supervision, vaccinations were delivered to dogs intra-muscularly in the longissimus muscle (loin area), although subcutaneous vaccination is also acceptable. Follow up booster injections were administered on the contra-lateral side. No pain or adverse reactions were observed at the injection sites. In some cases boosters were administered subcutaneously with equivalent results.

Antibody titers. Blood was drawn from each dog weekly, and serum antibody titers were determined using an enzyme linked immunosorbant assay (ELISA). Adjacent wells of a microwell plate were coated with 2 μg pZP, and incubated for 6 hours. The wells were then blocked with 5% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) in TBST (Tris-buffered saline + 5% Tween-20) and incubated overnight. Wells were then loaded with the primary antibody (canine serum) in TBST at a 1:500 and 1:1,000 dilution and incubated for 4 hours. The wells were then washed and loaded with 50 μl of the

secondary antibody (rabbit anti-dog IgG) and incubated for 2 hours. Color change was observed after the addition of *p*-nitrophenyl phosphate for 30 minutes and the reaction terminated by the addition of 3 M NaOH. The optical density was read at a 405-492 nm range on a Spectramax spectrophotometer. The dogs pre-immune serum served as the negative controls.

The ELISA trials (Fig. 2) revealed that there was a similar antibody profile in all eight dogs (experimental and clinical) characterized by a significant rise in antibody titers between the first and second booster. Antibody levels rose slightly after the initial vaccination and then significantly ($p<0.05$) after the first and second boosters. The rise in titer was the greatest in the clinical trial dogs (trials 2-5). These data clearly show that the there is a significant immune response to the pZP vaccine and synthetic adjuvant.

Immunochemical and histochemical studies. The nature and extent of the immune response was investigated by performing histological and immunohistochemical studies on ovarian sections of the experimental dogs. Histological evaluation revealed that all tertiary follicles were significantly invaded by neutrophils. In these follicles all of the oocyte-granulosa cell complexes had been disrupted, and there were virtually no immunoreactive canine zona pellucida glycoproteins remaining in the ovary. Primary and secondary oocytes showed vacuolization and neutrophil infiltration.

The immunological response was further investigated by treating formalin fixed, paraffin embedded ovarian sections with anti-pZP antibodies raised in rabbits against highly purified pZP, and incubating for 1 hour. The sections were then treated with biotin-conjugated anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO), followed by avidin-conjugated horseradish peroxidase (Sigma Chemical Co., St. Louis, MO). Finally, the sections were stained with diaminobenzidine and counterstained with Mayer's hematoxylin. In

the vaccinated dogs, the integrity of all ovarian follicles was found to have been breached, and no immunodetectable zona material was present on the ovarian sections. In contrast, normal dog ovaries have distinct oocytes with a zona pellucida. These results suggest that canine sterility was achieved as a result of destruction of all ovarian follicles.

None of the vaccinated dogs have shown any abnormal estrus cycles. Moreover, the vaccine is effective in pre-pubertal dogs, suggesting that if dogs are sterilized before their first estrus, their chances of developing mammary cancer or uterine infections are virtually zero.

WHAT IS CLAIMED IS:

1. A fertility impairing vaccine comprising an antigen comprising substantially pure, nonrecombinant zona pellucida glycoprotein, or an antigenic fragment thereof.

2. The fertility impairing vaccine of claim 1 wherein the zona pellucida glycoprotein is porcine zona pellucida glycoprotein.

3. The fertility impairing vaccine of any preceding claim further comprising an immunological adjuvant.

4. The fertility impairing vaccine of claim 3 wherein the immunological adjuvant comprises synthetic trehalose dicorynomycolate.

5. The fertility impairing vaccine of any preceding claim wherein the vaccine further comprises squalene oil.

6. The fertility impairing vaccine of any preceding claim which is an immunosterilant vaccine.

7. A method for impairing the fertility of an animal comprising administering to the animal the vaccine of any of the preceding claims wherein the vaccine is administered in a manner and an amount effective to cause fertility impairment in the animal.

8. The method of claim 7 wherein the vaccine causes temporary, reversible infertility in the animal.

9. The method of claim 7 wherein the vaccine causes permanent, irreversible infertility in the animal.

10. The method of claims 7, 8 or 9 wherein the animal is a carnivore.

11. The method of claim 10 wherein the carnivore is a dog or a cat.

12. The method of claim 11 wherein the carnivore is a dog.

13. The method of claim 12 wherein administration of the vaccine does not cause abnormal estrus cycles in the dog.

AMENDED CLAIMS
[received by the International Bureau on 14 June 1999 (14.06.99); original claim 10 cancelled; original claims 1 and 7-9 amended; claims 11-13 renumbered as claims 10-12 amended; remaining claims unchanged (2 pages)]

1. A fertility impairing vaccine for use in carnivores comprising an antigen comprising substantially pure, nonrecombinant zona pellucida glycoprotein or an antigenic fragment thereof.

2. The fertility impairing vaccine of claim 1 wherein the zona pellucida glycoprotein is porcine zona pellucida glycoprotein.

3. The fertility impairing vaccine of any preceding claim further comprising an immunological adjuvant.

4. The fertility impairing vaccine of claim 3 wherein the immunological adjuvant comprises synthetic trehalose dicorynomycolate.

5. The fertility impairing vaccine of any preceding claim wherein the vaccine further comprises squalene oil.

6. The fertility impairing vaccine of any preceding claim which is an immunosterilant vaccine.

7. A method for impairing the fertility of a carnivore comprising administering to the carnivore the vaccine of any preceding claim, wherein the vaccine is administered in a manner and an amount effective to cause fertility impairment in the carnivore.

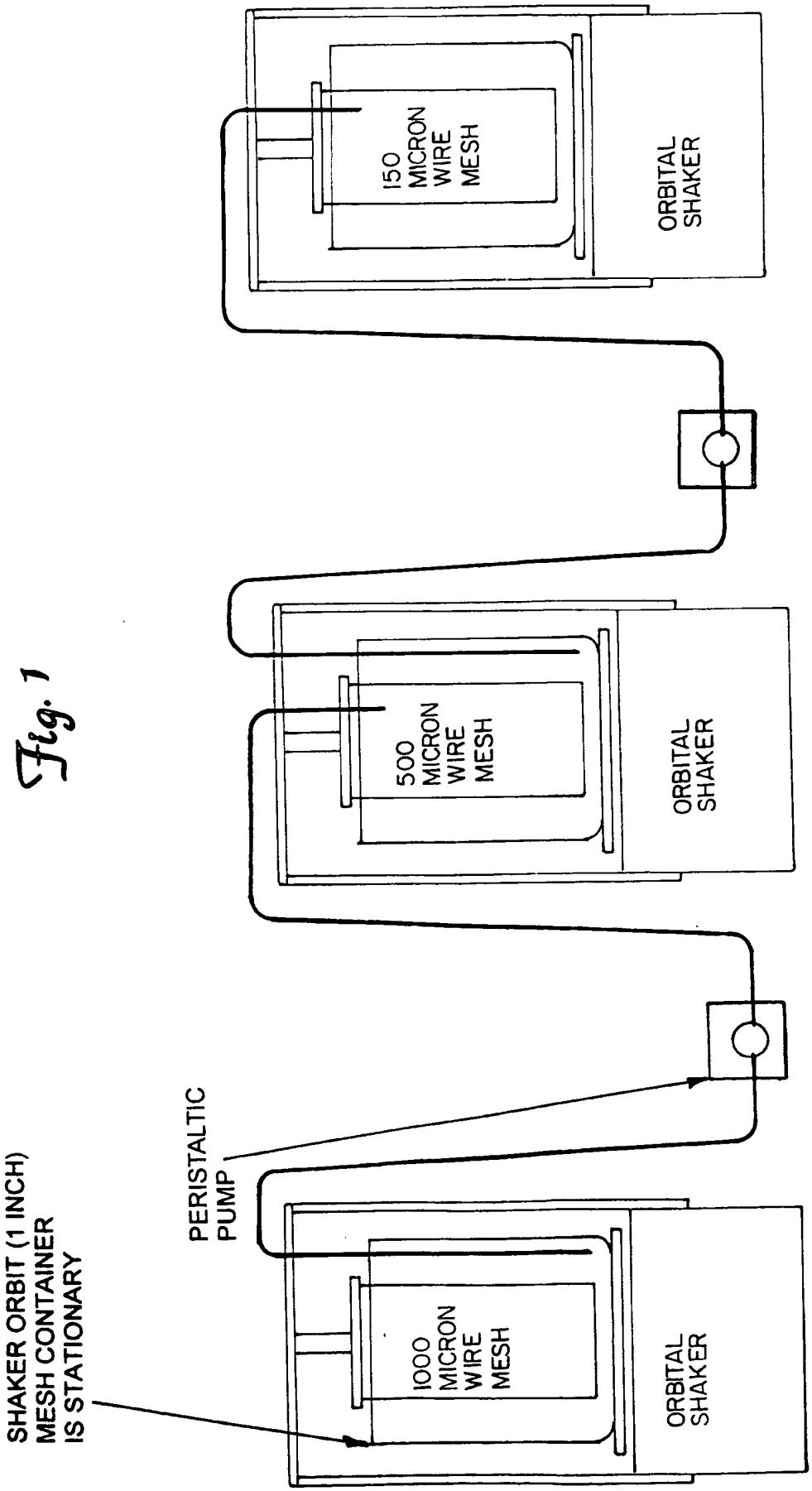
8. The method of claim 7 wherein the vaccine causes temporary, reversible infertility in the carnivore.

9. The method of claim 7 wherein the vaccine causes permanent, irreversible infertility in the carnivore.

10. The method of claims 7, 8, or 9 wherein the carnivore is a dog or a cat.

11. The method of claim 10 wherein the carnivore is a dog.

12. The method of claim 11 wherein administration of the vaccine does not cause abnormal estrus cycles in the dog.


SHAKER ORBIT (1 INCH)
MESH CONTAINER
IS STATIONARY
PERISTALTIC
PUMP
1000
MICRON
WIRE
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SHAKER
500
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WIRE
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150
MICRON
WIRE
MESH
ORBITAL
SHAKER

Fig. 1

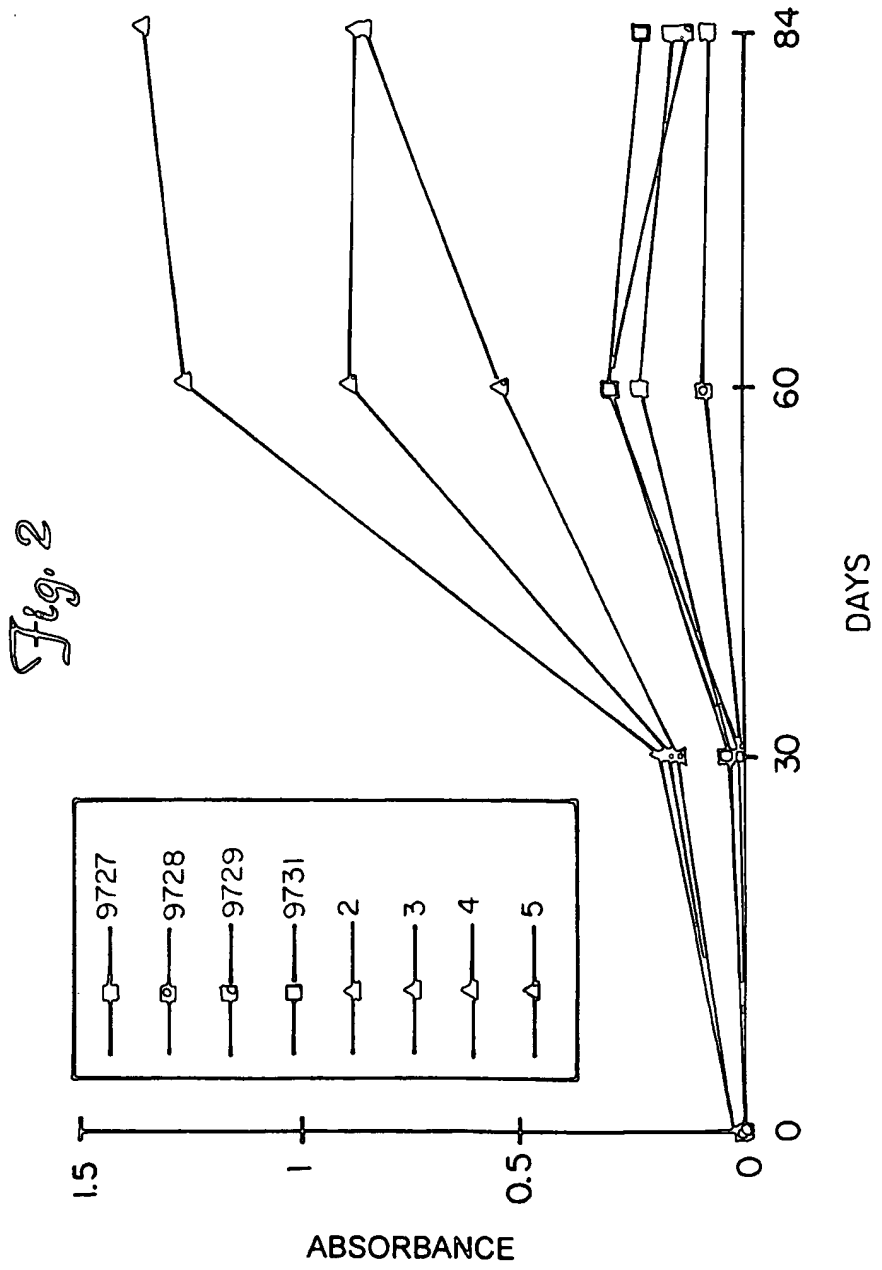

Fig. 2

INTERNATIONAL SEARCH REPORT

Inter: nal Application No
PCT/US 98/27658

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 11019 A (ZONAGEN INC) 26 May 1994 see page 1, line 10-15 see examples 5-8 ---	1-13
X	WO 89 03399 A (ZONAGEN INC) 20 April 1989 see page 4, line 10-22 see page 5, line 30 - page 6, line 5 see page 21, line 19-21 see page 15, line 19-21 see example 6 ---	1-13
X	WO 93 14786 A (UNIV COLORADO RES) 5 August 1993 see page 4, line 13 - page 5, line 2 see examples 1,6 --- -/--	1-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1999	28/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Covone, M

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 98/27658

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DIETL J. ET AL: "Immunogenic potency of the zona pellucia" EXPERIENTIA , vol. 38, 1982, pages 502-503, XP002100172 see the whole document	1-9
X	SACCO A G ET AL.: "Heteroimmunization of squirrel monkeys (Saimiri sciureus) with a purified porcine zona antigen (PPZA): immune response and biologic activity of antiserum" FERTILITY AND STERILITY, vol. 39, no. 3, March 1983, pages 350-358, XP002100173 see abstract see page 355, right-hand column, line 5-14 see table 1	1-9
X	PATERSON M ET AL: "Analysis of the contraceptive potential of antibodies against native and deglycosylated porcine ZP3 in vivo and in vitro" BIOLOGY OF REPRODUCTION , vol. 46, 1992, pages 523-534, XP002100174 see table 1 see page 530, right-hand column, line 14 - page 532, left-hand column, line 34	1-9
A	EAST I J ET AL: "MONOCLONAL ANTIBODIES TO THE MAJOR PROTEIN OF THE MURINE ZONA PELLUCIDA: EFFECTS ON FERTILIZATION AND EARLY DEVELOPMENT" DEVELOPMENTAL BIOLOGY, vol. 104, no. 1, July 1984, pages 49-56, XP002052324	1-9

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/27658

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7-13
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 7-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/ composition.

Claims Nos.: 7-13

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/US 98/27658

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9411019 A	26-05-1994	AU 675269 B	30-01-1997
		AU 5680094 A	08-06-1994
		CA 2127531 A	26-05-1994
		EP 0634936 A	25-01-1995
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WO 8903399 A	20-04-1989	US 4996297 A	26-02-1991
		AU 630862 B	12-11-1992
		AU 2536088 A	02-05-1989
		EP 0396552 A	14-11-1990
		EP 0599822 A	01-06-1994
		IN 167608 A	24-11-1990
		JP 3502571 T	13-06-1991
		US 5820863 A	13-10-1998
WO 9314786 A	05-08-1993	AU 3611793 A	01-09-1993